Dear Sir:

In view of the current interest in the effects, or possible effects, of asbestos introduced to the body both by inhalation and ingestion, and the interest that this has aroused in the lay press as well as the scientific press, we believe that it is timely to suggest a critical reappraisal of the results obtained using Soluene and KOH as tissue digestants in the examination of tissue samples for asbestos.

The reagent Soluene is widely used as a tissue digestant, particularly in radioimmunoassay work. Its use has also been reported for the digestion of tissue prior to the determination of its asbestos content (*1*). Our recent experience in this context indicated that Soluene is not suitable as a tissue digestant for the determination of asbestos in tissue and that the data obtained by such a method must be regarded as suspect.

As part of a study of the effects of asbestos introduced into the body system intravenously, we have been analyzing tissue samples from experimental animals. Although, initially, our control samples showed no detectable asbestos fibers, later samples in the series showed considerable amounts (Table 1), in some cases higher than the levels exhibited by similar organs in animals which had been injected intravenously with a chrysotile suspension. The previous work (*1*) also reported abnormally high levels in the control animals. We, therefore, attempted to establish the origin of this contamination by examining all solvents and solutions used in the preparation of the samples. Only the Soluene showed any asbestos contamination and we therefore suspected that it has been filtered through an asbestos filter. This was confirmed by the suppliers of the Soluene, who stated that the reagent had indeed been filtered through an asbestos filter at one stage in its manufacture. As no asbestos could be detected in any of the other reagents which we were using, it is clear that the use of Soluene to prepare tissue samples in this manner does introduce an artificial asbestos content to the preparation. In our experience with Soluene, the extent of this contamination was variable and unpredictable, thereby invalidating any conclusions which might be reached on the asbestos levels of tissues prepared by this technique.

KOH has similarly been used as a digestant for tissue in the preparation of samples for asbestos examination. Published data on the occurrence of asbestos in caustic solutions (*2*) where the caustic has been prepared by a diaphragm process suggests that there is also a potential for contamination by asbestos of tissue samples digested by KOH.

A sample preparation method using low temperature ashing of the tissue in an oxygen plasma has been found to be effective, yielding clean preparations in which the asbestos fibers may readily be identified and measured. Replicate samples on aliquots of tissue removed from the lung of an asbestos injected animal have shown good reproducibility. No asbestos was observed in similar organs removed from control animals which had not been injected with asbestos.

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1. Cunningham, H. M., and Pontefract, F. D. Nature 243: 352 (1973).
2. Beaman, D., and File, D. M. Anal. Chem. 48: (1976).

Table 1. Asbestos content in control samples of rat tissue prepared by Soluene or by low temperature ashing.

	No. of fibers/g of tissue	
Tissue type	Soluene treatment	Low temperature ashing
Muscle	3×10^6	BDL[a]
Lymph nodes	3.5×10^6	BDL
Liver	1.8×10^6	BDL
Spleen	1.6×10^6	BDL
Lung	24×10^6	BDL
Heart	0.25×10^6	BDL

[a] BDL = below detection limit; detection limits in the range $0.02\text{-}0.2 \times 10^6$ fibers/g.

Dear Sir:

In reply to the letter of Grieger and Stewart, each batch of Soluene used in our experiments (*1*) was checked in advance for asbestos contamination using reagent blank determinations. Only occasionally was any asbestos detected and in such cases the Soluene was either centrifuged to lower the asbestos below our detection limit or the batch was used for other work. So little asbestos

was detected in these blank determinations that we were not certain whether it came from the Soluene or other sources. You may note that the blood of control rats which was analyzed with the use of Soluene did not contain detectable amounts of asbestos and zero fibers per gram was reported.

We have since repeated this work (as yet unpublished) in an experiment in which asbestos was fed to rats and the tissues analyzed by a low temperature ashing technique similar to that reported for fecal asbestos (*2*). Essentially similar results were obtained as in the original work with significantly higher levels of asbestos fibers found in treated than in control animals.

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1. Pontefract, R. D., and Cunningham, H. M. Nature, 243: 352 (1973).
2. Cunningham, H. M., Pontefract, R. D., and O'Brien, R. C. J. Toxicol. Environ. Health 1: 377 (1976).

Dear Sir:

Polychlorinated biphenyls (PCBs), ubiquitous environmental pollutants, are usually assayed by gas chromatography of cleaned-up extracts with electron capture detection. Quantitation is relative to some arbitrary standard, usually one of the commercial PCB mixtures (Aroclors, Clophens, etc.). The mixtures recovered from environmental samples (soil, feces, various animal tissues, milk and the like) almost never match the PCB standard mixture in composition, but the commercial mixture best approximating the unknown in general distribution of peaks on the chromatograms is necessarily selected as quantitation reference.

Although it is well known (*1,2*) that electron capture detectors respond differently to different PCBs, the assumption is usually made that the errors in comparing an unknown mixture with a more-or-less similar standard mixture will cancel out. Accordingly, the literature is replete with data on "PCB content" of all sorts of environmental samples (*3*). In a few cases (*4*), the numbers reported were roughly confirmed by perchlorination to the single compound decachlorobeiphenyl (which can be accurately quantitated with an electron capture detector), but in the vast majority of cases, there has been no confirmation.

To illustrate the unreliability of the more common direct gas chromatography of PCBs versus an Aroclor standard, we performed the following simple experiment. A 1-g portion of Aroclor 1260 was chromatographed on 50 g of Florisil PR, eluting with ligh petroleum ether. Under these conditions, the PCBs tend to "tail". The portion eluting between 200 and 300 ml of petroleum ether weighed about 4 mg and showed all of the same peaks as the original Aroclor 1260 during gas chromatography. However, the relative proportions of the peaks differed from those of stock 1260.

Three preparations were supplied for gas chromatographic analysis; the original Aroclor 1260, for use as reference standard, cottonseed oil spiked with 11.0 μg Aroclor 1260/g oil, and cottonseed oil spiked with 10.0 μg Aroclor 1260 "tail" (simulated environmental sample)/g oil. The analyst was simply instructed to analyze the two cottonseed oil samples for "total PCB content", using the Aroclor 1260 as reference standard. He did not know what PCB mixture or what concentration range to expect to find in the oil (i.e., a single-blind experiment).

Work-up of the samples was typical of procedures used for fatty materials, involving extraction into hexane:benzene, 5:1 (v/v), partitioning with sulfuric acid to remove lipids, drying with sodium sulfate-sodium carbonate mixture, and chromatography on Florisil. In every case, elution (with hexane:diethyl ether, 94:6) was shown to be sufficient to remove all of the PCBs from the column (no PCBs were seen in a subsequent elution with hexane:diethyl ether, 85:15). Gas chromatography was routine, with a Varian 2100 gas chromatograph, Sc^3H electron capture detector, and a glass column (2 mm ID × 6 ft) of 1.5% OV-17 + 1.95% QF-1 on 80/100 Gas Chrom Q. Each sample was analyzed five times. Quantitation was done in two ways: by summing all of the peak areas attributable to PCBs, and by summing the areas of four conspicuous peaks selected from chromatograms of the Aroclor 1260 standard. Both methods are commonly used in different